# Phylogenetic Placements of *Loxoscaphe thecifera* (Aspleniaceae) and *Actiniopteris radiata* (Pteridaceae) Based on Analysis of *rbcL* Nucleotide Sequences

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ABSTRACT.—Nucleotide sequences of the chloroplast-encoded *rbcL* gene were determined for *Loxoscaphe thecifera* and *Actiniopteris radiata* and used in maximum parsimony cladistic analyses to determine their phylogenetic positions in the context of a broad range of advanced fern taxa. *Loxoscaphe* nested firmly within the Aspleniaceae, and *Actiniopteris* was placed with *Onychium* in the Pteridaceae. To help resolve conflicting contemporary treatments that either subsume *Loxoscaphe* species within *Asplenium* or segregate them as an independent genus, the *rbcL* sequence of *L. thecifera* was subjected to a more focused analysis involving all *rbcL* sequences available to represent the taxonomic diversity of Aspleniaceae. *Loxoscaphe thecifera* was sister to *Asplenium griffithianum*+*prolongatum*, robustly and surprisingly nested within the clade of *Asplenium* species recognized as *Asplenium* section *Thamnopteris*, a group accepted by some as the segregate genus *Neottopteris*. These results indicate that there is presently no phylogenetic justification for accepting *Loxoscaphe* as a genus independent of *Asplenium*. Similarly *Actiniopteris radiata*, recently moved from the cheilanthoid to the taenitidoid group of Pteridaceae, was subjected to a more focused analysis in the context of an expanded set of cheilanthoid and taenitidoid species that included the first use of an *rbcL* sequence from the genus *Anogramma* and newly sequenced species of *Onychium* and *Pteris*. *Actiniopteris* is robustly grouped with two *Onychium* species in a clade sister to traditional taenitidoids and deeply separated from the cheilanthoids, supporting affinities previously suggested by spore morphology.

Loxoscaphe T. Moore is a genus of epiphytic species segregated from *Asplenium* L. by many contemporary authors (e.g., Crabbe et al., 1975; Pichi Sermolli, 1977; Smith, 1981; Mickel and Beitel, 1988; Lellinger, 1989), although others (Tryon and Tryon, 1982; Kramer and Viane, 1990; Tryon and Stolze, 1993) reject this segregation, instead subsuming *Loxoscaphe* species within the genus *Asplenium*. Mickel and Beitel (1988) expressed the segregationist view in stating that "*Loxoscaphe* is a genus of four or five species, one in the neotropics, two in Africa (one sometimes considered only varietally distinct from the neotropical species), and two in the South Pacific and Indonesia. Although it is most closely allied to and sometimes placed in synonymy under *Asplenium, Loxoscaphe* superficially more closely resembles *Odontosoria* in the pocketlike sori terminal on the narrow blade segments, but the small fronds, heavy texture, and epiphytic habit readily distinguish it from that genus." Its sori in pouches near the margin of the leaf segments (Fig. 1) give it the appearance of a *Davallia*, in which genus most of the older species of *Loxoscaphe* were first described, as noted by Copeland (1947). In commenting on the generic placement of *Loxoscaphe thecifera* (Kunth) T. Moore (as *Asplenium*) in their treatment of the ferns of Peru, Tryon and Stolze (1993)
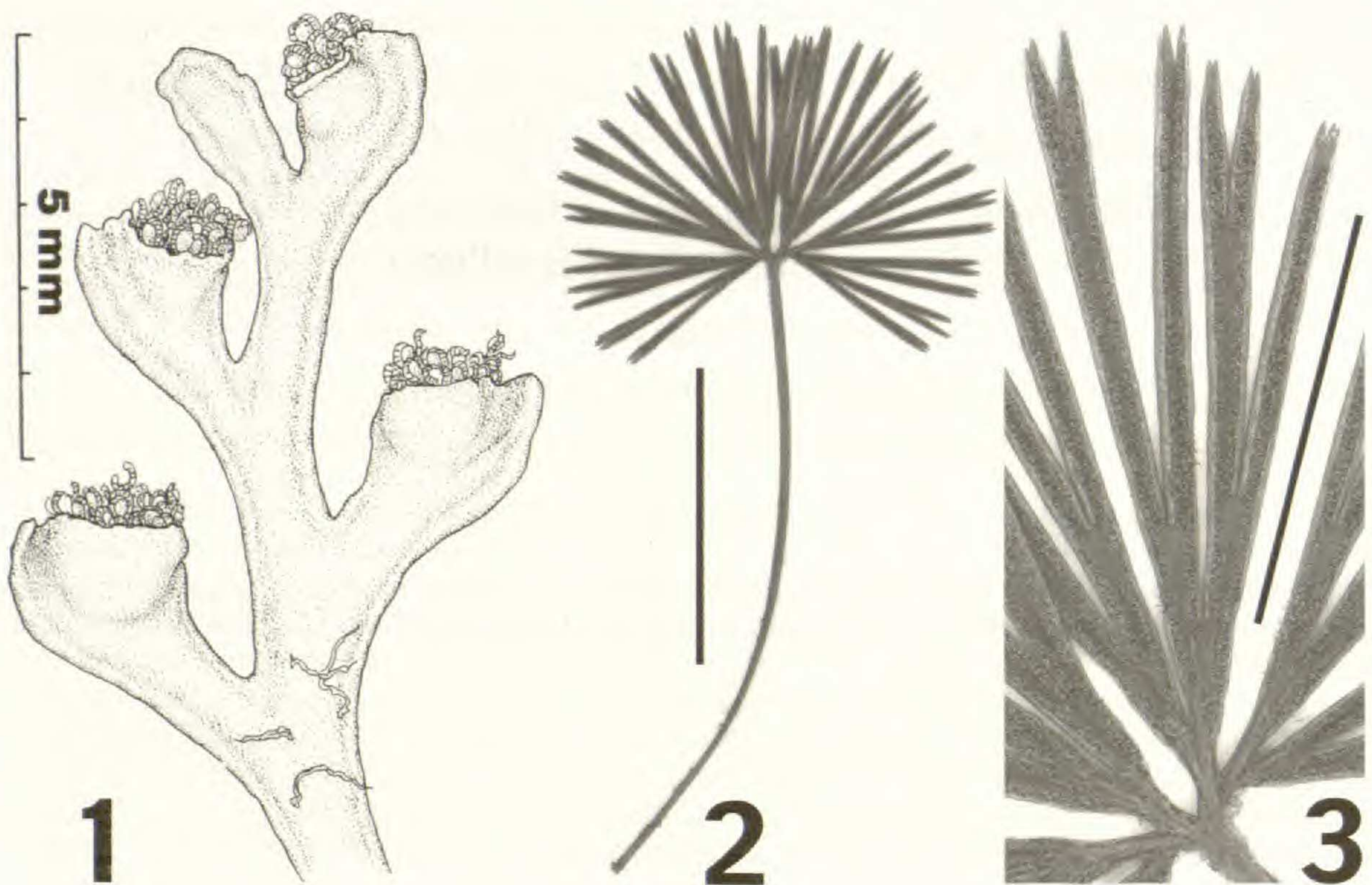
FIGS. 1–3.   Illustrations of *Loxoscaphe thecifera* and *Actiniopteris radiata*. 1. Apex of a pinna of *Loxoscaphe thecifera* drawn from *Holm-Nielson et al. 4110*, Ecuador (F) to show the pouch-like indusia characteristic of *Loxoscaphe*. Reproduced from Tryon and Stolze (1993) with permission from the Field Museum of Natural History. 2. Adaxial view of a sporophyll from a cultivated plant of *Actiniopteris radiata* (*Gastony 12–97–102* IND from Tanzania), showing the distinctive, flabellate lamina of this xeromorphic fern. Scale bar = 5 cm. 3. Magnified view of fascicle of segments from the right half of the lamina in Fig. 2, showing continuous, membranous, marginal indusia partly covering the submarginal sori. Scale bar = 2 cm.

noted that the group of *Asplenium* species often placed in the genus *Loxoscaphe* are quite distinctive in their indusia, which are generally pouch- or cup-shaped and occur near the segment margin. They noted that by means of this indusial character state, *Asplenium theciferum* (Kunth) Mett. cannot be confused with any other *Asplenium* in Peru, their area of coverage. Their retention of the *Loxoscaphe* group within *Asplenium* was influenced by their observation that the sori of a few Old World species in the group intergrade to a more typical *Asplenium* sorus.

The global analysis of fern phylogeny by Hasebe et al. (1995) based on *rbcL* nucleotide sequences did not include a representative of the *Loxoscaphe* species group, but the results of that study do provide a context in which the generic relationships of *Loxoscaphe* can now be assessed. Doing that is one goal of this paper. To focus the reader's attention on *Loxoscaphe* and other relevant groups sometimes proposed for generic segregation from *Asplenium*, the following text and figures 5 and 6 refer to these taxa by their names in segregate genera. This is simply an attention-focusing device and does not signal our taxonomic conclusions, which are sometimes contrary to the seg-

regate nomenclature. Alternative nomenclatures for these groups are noted in the lists of species in MATERIALS AND METHODS and in Table 1.

Actiniopteris Link was regarded as an Old World genus of cheilanthoid affinities by Tryon and Tryon (1982) who placed it (as *Actinopteris*) in Pteridaceae tribe Cheilantheae. Later, Tryon et al. (1990) shifted *Actiniopteris* from cheilanthoid to taenitidoid affinity by placing it in Pteridaceae subfamily Taenitidoideae, without explanation. *Actiniopteris radiata* (Sw.) Link, with an unusual fan-shaped lamina (Fig. 2) bearing linear, submarginal sori with continuous, membranous, marginal indusia (Fig. 3), is widespread within the Afro-Indian distribution of its genus (Tryon et al., 1990). It occurs, for example, throughout tropical Africa where hot and dry conditions prevail (Jacobsen, 1983). Continuing the ongoing efforts of the first author to decipher phylogenetic relationships among the lineages of cheilanthoid ferns (Gastony and Rollo, 1995, 1998), a second goal of the present study is inferring the subfamilial affinities of *Actiniopteris* on the basis of *rbcL* data.

The first author encountered epiphytic plants of *Loxoscaphe thecifera* on the rim of the Ngorongoro Crater and terrestrial plants of *Actiniopteris radiata* in fissures of the limestone cap bordering Olduvai Gorge, both during a recent trip to Tanzania. Materials suitable for DNA-level analyses of these species were obtained. The resulting *rbcL*-based phylogenetic analyses determine the phylogenetic relationships of these species and provide a fresh perspective for addressing the generic disposition of *Loxoscaphe*.

MATERIALS AND METHODS

Total genomic DNA of *Actiniopteris radiata* and *Loxoscaphe thecifera* was extracted by the DNeasy method (Qiagen, Inc., Valencia, CA) from silica-gel dried leaves of sporophytes collected in nature (Table 1), purified with the Elu-Quick DNA purification kit (Schleicher & Schuell, Keene, NH), and quantified to 20 ng/μl with a Hoeffer Scientific fluorometer. DNA of the other species in Table 1 was obtained as follows: *Anogramma lorentzii* from gametophytes grown from spores of the Brazilian sporophyte voucher, *Pteris cretica* from fresh, field-collected sporophytes, and *Onychium lucidum* from sporophytes grown from spores of the Chinese voucher. Polymerase Chain Reaction (PCR) amplification of the *rbcL* gene was as reported in Gastony and Rollo (1995) except that for *Loxoscaphe thecifera* the hot start PCR method of Gastony and Ungerer (1997) was used with the following specifications: 0.8 μl @20 ng/μl of genomic template DNA was added to 8.62 μl distilled H_2O, 3.6 μl of Master Mix (see components below), 0.24 μl of 10 μM forward primer 1F, and 0.24 μl of 10 μM reverse primer 1351R (primers 1F and 1351R as in Gastony and Rollo, 1995). In PCR, these reagents were heated to 96°C for 5 min to denature the templates before adding 1.5 μl Taq polymerase ("hot start") during a 5 min period at 72°C. The final concentration of the hot start Master Mix was 2 mM $MgCl_2$, 30 mM tricine, 50 mM KCl, 100 μM of each dNTP, and 5% acetamide. In all cases PCR was carried out in an MJ Research Thermal Cycler programmed as follows (after the initial 96°C denaturation or

TABLE 1. Sources of material and GenBank accession numbers for *rbcL* sequences of taxa used in this study. Species for which data were published in the Appendix of Hasebe et al. (1995), Table 2 of Murakami et al. (1999a), or Table 1 of Murakami et al. (1999b) are not repeated here (see Materials and Methods for lists of those species). All collections below are vouchered at IND.

Species	Provenance	Collection (Voucher)	GenBank accession no.	Figures in this study
Actiniopteris radiata (Sw.) Link	Olduvai Gorge, Tanzania	*Gastony 12-97-102*	AF336100	4, 7
Anogramma lorentzii (Hieron.) Diels	Rio Grande do Sul, Brazil	*Sehnem s.n.,* 10/10/72	AF336102	7
Asplenium theciferum (Kunth) Mett.				
≡ *Loxoscaphe thecifera* (Kunth) T. Moore	Ngorongoro Crater, Tanzania	*Gastony 12-97-101*	AF336099	4, 5, 6
Cheilanthes californica (Hook.) Mett.	California, U.S.A.	*Kirkpatrick s.n.*	AF336101	4, 7
Onychium lucidum Spreng.	Yunnan, China	*Li & Xiang S-8L*	AF360359	7
Pityrogramma calomelanos (L.) Link	Oaxaca, Mexico	*Yatskievych & Gastony 89-251*	AF336103	7
Pityrogramma trifoliata (L.) R. M. Tryon	Oaxaca, Mexico	*Yatskievych & Gastony 89-252*	AF336104	7
Pteris cretica L.	Hidalgo, Mexico	*Yatskievych & Gastony 89-208*	AF360360	7

after the hot start addition of Taq): 40 cycles of 94°C for 1 min, 45°C for 1 min, 72°C for 2 min, followed by a 6 min final extension at 72°C and storage at 4°C. PCR yielded a 1381 base pair fragment of the *rbcL* gene, including the forward and reverse primer regions. With the suite of forward and reverse sequencing primers listed in Table 2 of Gastony and Rollo (1995), we were able to read 1325 bp of sequence between the PCR primers in both directions. Sequencing was carried out with an ABI automated sequencer according to manufacturer's protocols, and the contigs were assembled and read with Sequencer™ 3.1.1 (Gene Codes Corporation, Ann Arbor, MI).

To determine the phylogenetic relationships of *Loxoscaphe thecifera* and *Actiniopteris radiata*, their 1325 bp *rbcL* sequences (Table 1) were analyzed cladistically with PAUP* 4.0b4a (Swofford, 1998) in three separate analyses as follows.

In the first analysis, the *Loxoscaphe* and *Actiniopteris* sequences were combined with those of a large subset of the species used in the global analysis of fern phylogeny of Hasebe et al. (1995) to determine where *Loxoscaphe* and *Actiniopteris* would probably have been positioned if their *rbcL* sequences had been included in that 1995 analysis. In reference to Fig. 3 of Hasebe et al. (1995), the *rbcL* sequences of all taxa from *Orthiopteris* through *Loxogramme* were obtained from GenBank (except for *Cornopteris crenulatoserrulata*, *Deparia bonincola*, and *Hypodematium crenatum*, which were provided by Mitsuyasu Hasebe) and used for the ingroup for this first analysis along with the new sequences of *Loxoscaphe* and *Actiniopteris* and that of *Cheilanthes californica* (Table 1), an American cheilanthoid sometimes segregated into the genus *Aspidotis* and shown by Gastony and Rollo (1998) to have Asian affinities. This broad spectrum of taxa includes dennstaedtioids, lindsaeoids, *Davallia*, taenitidoids, cheilanthoids, and other advanced fern taxa in order to permit free positioning of *Loxoscaphe* and *Actiniopteris* wherever their *rbcL* affinities might reasonably lie. As outgroup for this analysis the GenBank *rbcL* sequences of four taxa (*Loxoma*, *Cyathea* [represented by *Cyathea lepifera*, more appropriately named *Sphaeropteris lepifera* as in our Fig. 4], *Sphaeropteris*, and *Metaxya*) from the clade sister to this ingroup in Fig. 3 of Hasebe et al. (1995) were selected. Thus, 69 taxa (65 ingroup plus 4 outgroup, Fig. 4) were used in this first analysis with PAUP*4.0b4a (Swofford, 1998) on a Macintosh PowerPC G4 350MHz with the following specifications: maximum parsimony, heuristic search, all characters equally weighted, starting tree by stepwise addition, random addition sequence with 1000 replicates, random starting seed number generated by the program, TBR branch swapping, MulTrees, and steepest descent. Of the 1325 characters, 504 were parsimony-informative. The positions of *Loxoscaphe* and *Actiniopteris* determined by this first analysis permitted subsequent selection of appropriate new ingroups and outgroups for each of them in more detailed analyses of their respective relationships in the second and third analyses.

The first analysis placed *Loxoscaphe* with *Asplenium* and its relatives, and *Actiniopteris* in the clade of Pteridaceae plus Vittariaceae (Fig. 4). For a more focused analysis of the *rbcL* relationships of *Loxoscaphe* within Aspleniaceae
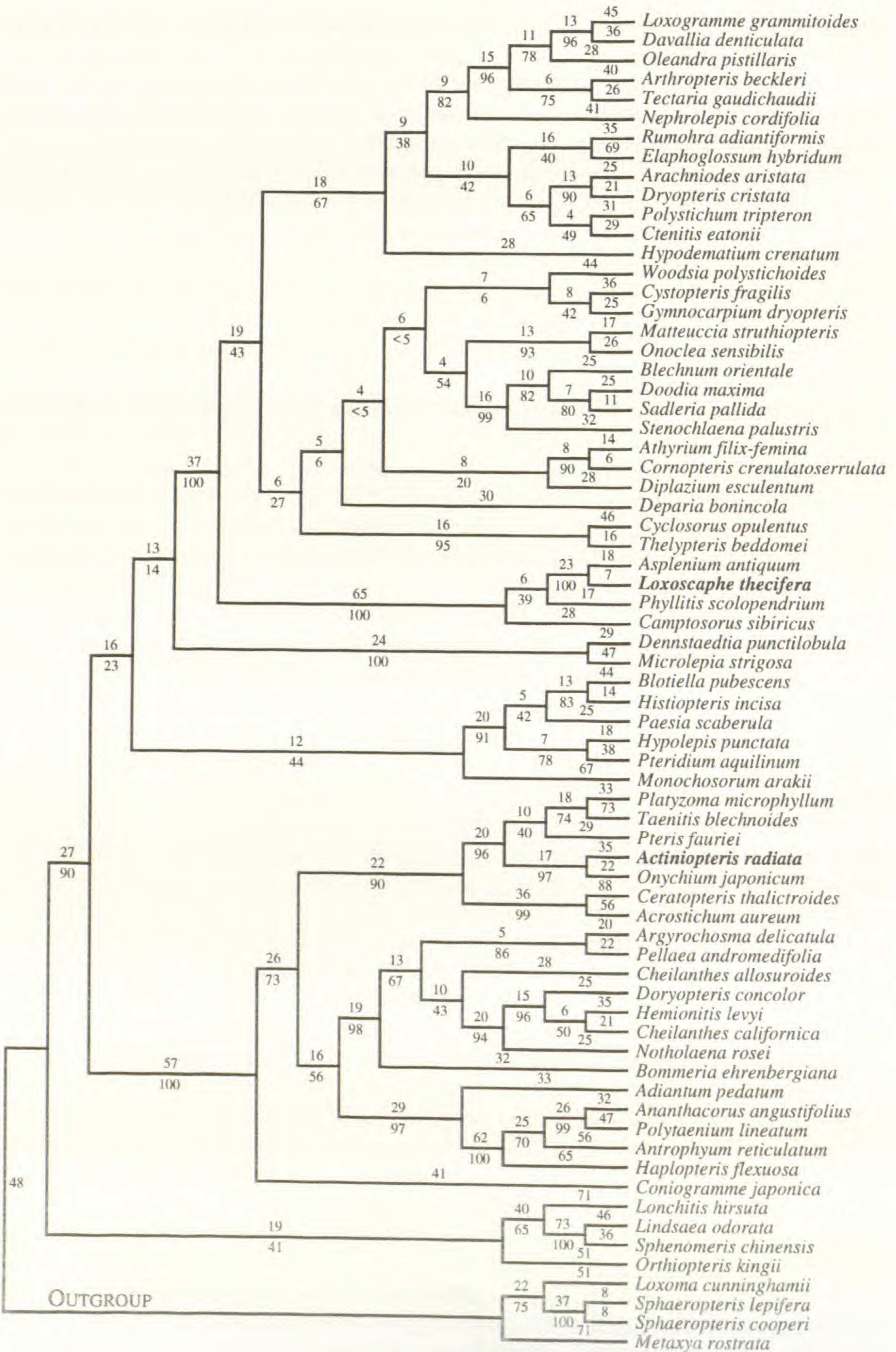
the clade from *Asplenium* through *Camptosorus* of Fig. 4 was therefore selected as a new ingroup, to which were added Aspleniaceae sequences available in GenBank (27 total ingroup taxa, Fig. 5), and a subset of seven taxa in the sister clade from *Loxogramme grammitoides* through *Thelypteris beddomei* (Fig. 4) was selected as the outgroup (Fig. 5). Most of these ingroup species were used in a recent *rbcL* study of the phylogeny of Aspleniaceae (Murakami et al., 1999b), the results of which are seen in Fig. 1 of that study. Use of generic names *Camptosorus, Neottopteris, Phyllitis,* and *Hymenasplenium* in discussing our results in Figs. 5 and 6 below facilitates comparison with Fig. 1 of Murakami et al. (1999b). *Boniniella ikenoi* of Murakami et al. (1999b) Fig. 1 is reported here under its name as a species of *Hymenasplenium, H. cardiophyllum.* Of the 1325 characters in this data set, 275 were parsimony-informative, probably reflecting the fact that the GenBank sequences of many of the *Asplenium* species used in the ingroup for Figs. 5 and 6 were much shorter than those generated in our lab, and their 5′ and 3′ end truncations relative to ours were treated as missing data.

Similarly, for a more focused analysis of the *rbcL* relationships of *Actiniopteris,* the clade from *Platyzoma microphyllum* through *Haplopteris flexuosa* of Fig. 4 was selected as a new ingroup. To these were added (Table 1) species of the putatively taenitidoid (Tryon and Tryon, 1982; Tryon et al., 1990) genera *Pityrogramma* (*P. calomelanos* and *P. trifoliata,* both previously used by Gastony and Rollo, 1998) and *Anogramma* (*A. lorentzii,* new here), and additional species of *Onychium, Pteris,* and *Cheilanthes* (*O. lucidum* and *P. cretica,* both new here; *C. lanosa* previously used by Gastony and Rollo, 1995, 1998), yielding 26 total ingroup taxa (Fig. 7, Table 1). As outgroup to this new *Actiniopteris* ingroup the sister clade *Coniogramme* of Fig. 4 was used, plus two basal taxa from the next most sister clade, viz. *Monachosorum arakii* and *Microlepia strigosa* (Fig. 4). Of the 1325 characters in the data set, 374 were parsimony-informative. Both of these more focused analyses were carried out in the same manner as the first analysis.

The bootstrap option in PAUP* was used to assess support for clades revealed in each of the three analyses. Bootstrapping for the first analysis was

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FIG. 4. Single most parsimonious tree placing *Loxoscaphe thecifera* and *Actiniopteris radiata* (bold) within the context of taxa seen in Fig. 3 of Hasebe et al. (1995), with the addition of *Cheilanthes californica.* Based on the findings in Fig. 3 of Hasebe et al. (1995), to which the topology of this tree is comparable, the bottom clade from *Loxoma* through *Metaxya* served as outgroup (with the name *Cyathea lepifera* of Hasebe et al. [1995] corrected to the more accurate designation *Sphaeropteris lepifera*). Crane et al. (1995) are followed in changing the name *Vittaria flexuosa* of Hasebe et al. (1995) to *Haplopteris flexuosa.* This tree was generated by a heuristic search with 1000 random addition sequence replicates used to obtain the starting trees. Numbers of synapomorphies supporting each clade and autapomorphic branch lengths for terminal taxa are indicated above the lines. Bootstrap percentages based on 1000 replicates of five random addition sequence replicates each are provided below the lines. The placements of *Loxoscaphe thecifera* and *Actiniopteris radiata* determined here directed choices of more focused ingroups and outgroups for the analyses reported in Figs. 5–7, as explained in the text.
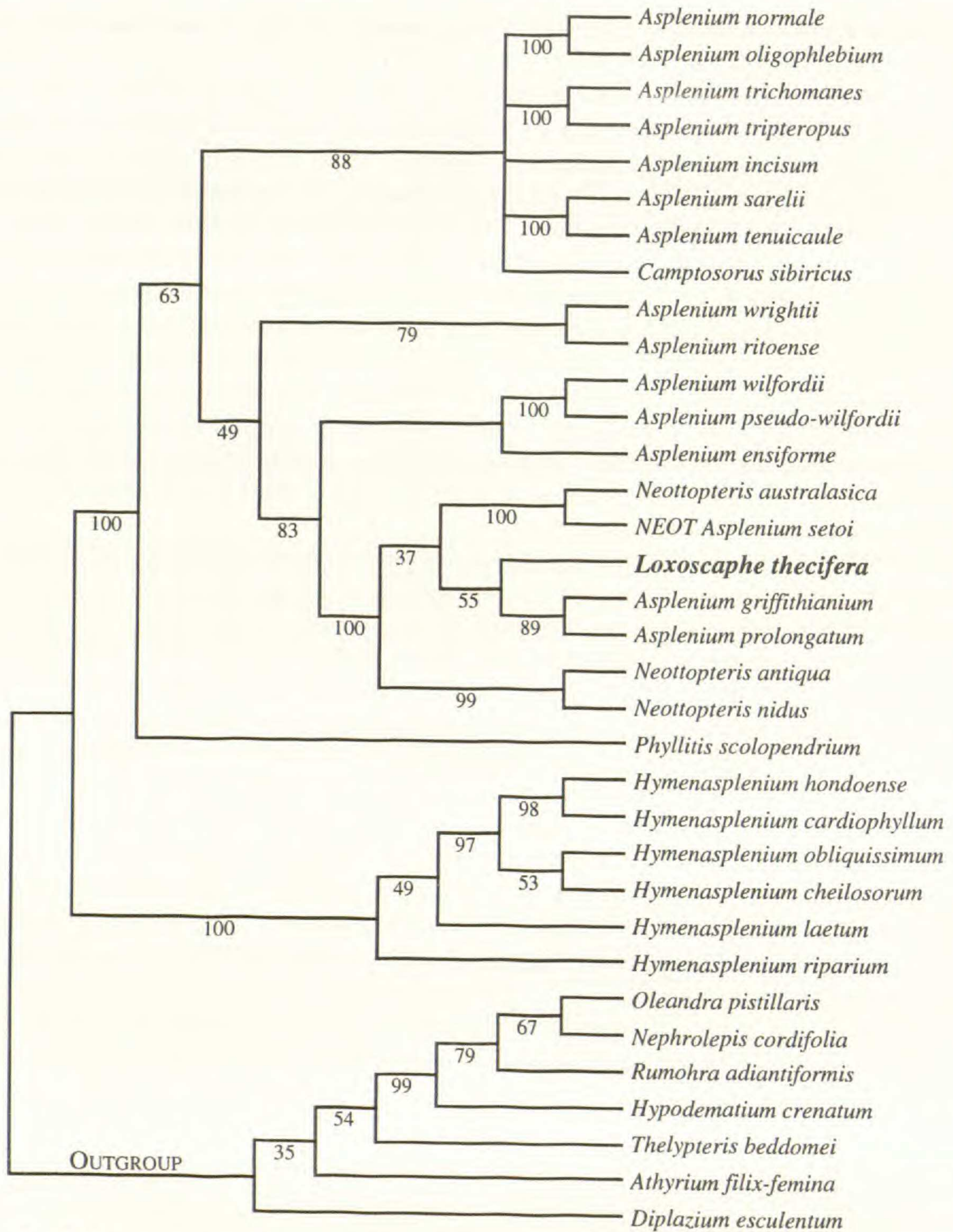
Fig. 5.   Strict consensus of 15 equally most parsimonious trees based on the new *rbcL* sequence of *Loxoscaphe thecifera* (bold) plus all *rbcL* sequences of species in Aspleniaceae presently available in GenBank. This tree was generated by a heuristic search with 1000 random addition sequence replicates used to obtain the starting trees. Taxa in the outgroup clade of *Oleandra* through *Diplazium* at the bottom were selected from clades sister to Aspleniaceae in Fig. 4. Bootstrap percentages based on 1000 replicates of five random addition sequence replicates each are provided below the lines. *NEOT* indicates that *Asplenium setoi* (segregated from *Asplenium australasicum* ≡ *Neottopteris australasica* by Murakami et al. [1999a]) would be placed in *Neottopteris* if that genus were accepted and the combination were made.
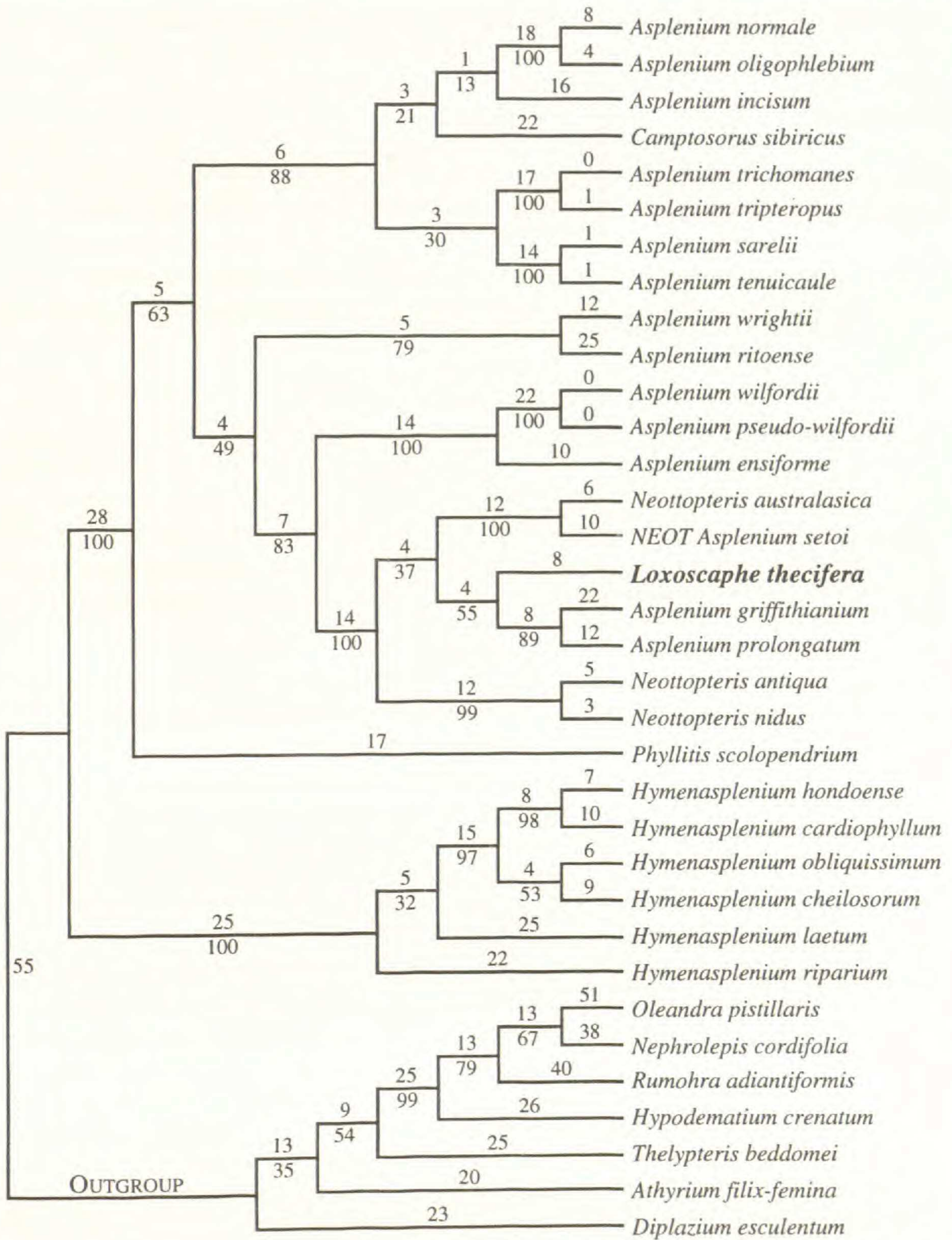
FIG. 6. One of the equally most parsimonious trees randomly selected from 15 represented by the consensus tree of Fig. 5 in order to present branch lengths (synapomorhies and autapomorphies) above the lines and bootstrap percentages as in Fig. 5 below the lines. The position of *Loxoscaphe thecifera* is given in bold. Branch lengths for the subclade *Neottopteris australasica* through *N. nidus,* in which *Loxoscaphe* is nested, are identical for all 15 equally parsimonious trees.
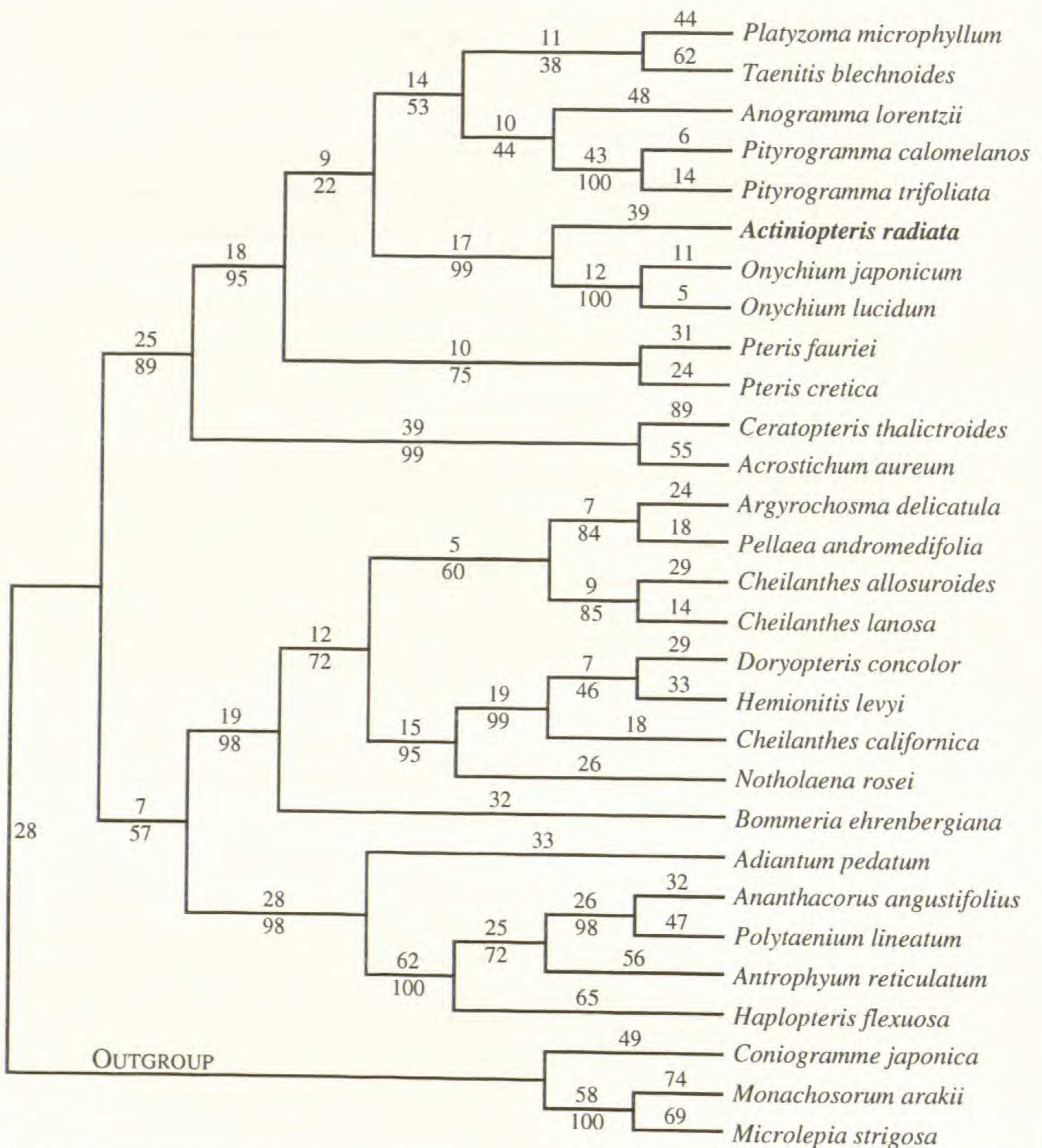
FIG. 7. Single most parsimonious tree based on new *rbcL* sequence of *Actiniopteris radiata* (bold), with other ingroup taxa drawn from the clade of *Platyzoma* through *Vittaria* in Fig. 4 supplemented with sequences of three putatively taenitidoid taxa (two *Pityrogramma* species previously used in Gastony and Rollo [1998] and *Anogramma lorentzii* used here for the first time) and with outgroup taxa *Coniogramme* through *Microlepia* drawn from clades progressively sister to the *Platyzoma* through *Vittaria* clade in Fig. 4. This tree was generated by a heuristic search with 1000 random addition sequence replicates used to obtain the starting trees. Number of synapomorphies supporting each clade and autapomorphic branch lengths for terminal taxa are indicated above the lines. Bootstrap percentages based on 1000 replicates of five random addition sequence replicates each are provided below the lines.

carried out via a maximum parsimony heuristic search of 1000 bootstrap replicates, with the starting tree obtained by stepwise addition based on a random addition sequence of five replicates, the random starting seed number generated by the program, and with TBR branch swapping, MulTrees, and steepest descent in effect. Bootstrapping for the two more focused analyses used the same settings and parameters as the first, except that for the more focused *Actiniopteris* analysis the 1000 bootstrap replicates were based on a random addition sequence of 10 replicates each.

To avoid unnecessary repetition of previously published voucher and GenBank data for species used in our analyses and figures, that information is not presented in Table 1 but can be found as follows (authors of names are given only when not found in the respective following references). See Appendix of Hasebe et al. (1995) for the following species: *Acrostichum aureum, Adiantum pedatum, Ananthacorus angustifolius, Antrophyum reticulatum, Arachniodes aristata, Argyrochosma delicatula, Arthropteris beckleri, Asplenium antiquum* (≡ *Neottopteris antiqua* (Makino) Masam.), *Asplenium nidus* (≡ *Neottopteris nidus* (L.) Hook.), *Asplenium ruprechtii* (≡ *Camptosorus sibiricus*), *Athyrium filix-femina, Blechnum orientale, Blotiella pubescens, Bommeria ehrenbergiana, Ceratopteris thalictroides, Cheilanthes allosuroides, Cheilanthes lanosa, Coniogramme japonica, Cornopteris crenulatoserrulata, Ctenitis eatonii, Cyathea lepifera* (see *Sphaeropteris lepifera* below), *Cyclosorus opulentus, Cystopteris fragilis, Davallia denticulata, Dennstaedtia punctilobula, Deparia bonincola, Diplazium esculentum, Doodia maxima, Doryopteris concolor, Dryopteris cristata, Elaphoglossum hybridum, Gymnocarpium dryopteris, Haplopteris flexuosa* (Fée) E. H. Crane as *Vittaria flexuosa* in Hasebe et al. (1995), *Hemionitis levyi, Histiopteris incisa, Hypodematium crenatum, Hypolepis punctata, Lindsaea odorata, Lonchitis hirsuta, Loxogramme grammitoides, Loxoma cunninghamii, Matteuccia struthiopteris, Metaxya rostrata, Microlepia strigosa, Monachosorum arakii, Nephrolepis cordifolia, Notholaena rosei, Oleandra pistillaris, Onoclea sensibilis, Onychium japonicum, Orthiopteris kingii, Paesia scaberula, Pellaea andromedifolia, Phyllitis scolopendrium, Platyzoma microphyllum, Polystichum tripteron, Polytaenium lineatum, Pteridium aquilinum, Pteris fauriei, Rumohra adiantiformis, Sadleria pallida, Sphaeropteris lepifera* (Hook.) R. M. Tryon as *Cyathea lepifera* in Hasebe et al. (1995), *Sphaeropteris cooperi, Sphenomeris chinensis* (note that the GenBank accession for this uses the synonym *Odontosoria chinensis*), *Stenochlaena palustris, Taenitis blechnoides, Tectaria gaudichaudii, Thelypteris beddomei, Vittaria flexuosa* (see *Haplopteris flexuosa* above), *Woodsia polystichoides.* See Table 2 of Murakami et al. (1999a) for the following (when more than one accession of a species is listed in that table, the GenBank accession number is given here to indicate which accession was used in our analysis): *Asplenium australasicum* AB013249 (≡ *Neottopteris australasica* J. Sm.), *Asplenium griffithianum, Asplenium setoi* AB013243 (segregated from *A. australasicum* by Murakami et al. [1999a]; would be a species of *Neottopteris* if that genus were accepted; combination apparently not yet made). See Table 1 of Murakami et al. (1999b) for: *Asplenium cardiophyllum* (≡ *Hymenasplenium*

cardiophyllum ≡ *Boniniella ikenoi*), *Asplenium cheilosorum* (≡ *Hymenas-plenium cheilosorum*), *Asplenium ensiforme*, *Asplenium hondoense* (≡ *Hymenasplenium hondoense*), *Asplenium incisum*, *Asplenium laetum* (≡ *Hymenasplenium laetum*), *Asplenium normale*, *Asplenium obliquissimum* (≡ *Hymenasplenium obliquissimum*), *Asplenium oligophlebium*, *Asplenium prolongatum*, *Asplenium pseudo-wilfordii*, *Asplenium riparium* (≡ *Hymenasplenium riparium*), *Asplenium ritoense*, *Asplenium sarelii*, *Asplenium tenuicaule*, *Asplenium trichomanes*, *Asplenium tripteropus*, *Asplenium wilfordii*, *Asplenium wrightii*.

RESULTS

The first analysis, designed to place *Loxoscaphe* and *Actiniopteris* within the context of the taxa in Fig. 3 of Hasebe et al. (1995), resulted in a single most parsimonious tree of 3573 steps (Fig. 4) whose general topology is similar to that of Fig. 3 in Hasebe et al. (1995). *Loxoscaphe thecifera* is positioned sister to *Asplenium antiquum* (23 synapomorphies and 100% bootstrap confidence) in the robustly supported (65 synapomorphies, 100% bootstrap value) clade of Aspleniaceae (*Asplenium antiquum* through *Camptosorus sibiricus*) near the center of the tree. This is far removed from genera noted in the literature (Mickel and Beitel, 1988; Copeland, 1947) as having superficially similar soral pouches—*Odontosoria* and its relatives toward the base of the tree (where *Odontosoria chinensis* is reported as *Sphenomeris chinensis*, as it was in Fig. 3 of Hasebe et al. 1995) and *Davallia* near the top. *Actiniopteris radiata* nests deeply within Pteridaceae+Vittariaceae (the clade from *Platyzoma microphyllum* through *Haplopteris flexuosa*) in the lower half of the tree, where its sister relationship to *Onychium japonicum* is strongly supported by 17 synapomorphies and a 97% bootstrap value. *Actiniopteris* is further placed by 22 synapomorphies and a 90% bootstrap value within the clade (*Platyzoma* through *Acrostichum*) that is sister to the clade containing the deeply separated and strongly supported cheilanthoids (*Argyrochosma delicatula* through *Bommeria ehrenbergiana*) that are themselves united by 19 synapomorphies and a 98% bootstrap value.

The more focused analysis designed to position *Loxoscaphe* within a context of all species of Aspleniaceae for which *rbcL* sequences were available in GenBank yielded 15 equally most parsimonious trees of 871 steps whose consensus topology (Fig. 5) is comparable to that in Fig. 1 of Murakami et al. (1999b). Fifteen equally most parsimonious trees were also produced by the similar but not identical data set used by Murakami et al. (1999b), with all variation both there and here attributable to unresolved polytomies in the clade from *Asplenium normale* through *Camptosorus sibiricus* (Fig. 5). The uncertainties of that clade, however, do not affect the positioning of *Loxoscaphe thecifera*, which nests within the fully resolved sister clade from *Asplenium wrightii* through *Neottopteris nidus* in all 15 equally most parsimonious trees. Within that clade, *Loxoscaphe* is placed within the subclade from *Neottopteris australasica* through *N. nidus* by 14 synapomorphies with a boot-

strap value of 100%, as seen in Fig. 6 of a tree randomly selected from the 15 in order to depict branch lengths. Branch lengths are identical for this *Loxoscaphe* subclade in all of the fifteen equally most parsimonius trees.

The more focused analysis designed to position *Actiniopteris* within an expanded database of taenitidoid and cheilanthoid ferns yielded a single most parsimonious tree of 1611 steps (Fig. 7). The *rbcL* data place *Actiniopteris radiata* sister to the two species of *Onychium* in a clade supported by 17 synapomorphies and a 99% bootstrap value. That robustly supported *Actiniopteris+Onychium* clade is in turn sister to one in which the intergeneric relationships are relatively weakly supported (53% bootstrap value), as is the entire clade from *Platyzoma* through *Onychium* (22% bootstrap value). Nevertheless, the *Actiniopteris+Onychium* clade is robustly embedded in clades from *Platyzoma* through *Pteris* (95% bootstrap value) and from *Platyzoma* through *Acrostichum* (89% bootstrap value) that are deeply separated from the robustly supported (98% bootstrap) cheilanthoid fern clade (*Argyrochosma delicatula* through *Bommeria ehrenbergiana*).

DISCUSSION

LOXOSCAPHE.—Results of the taxonomically broad first analysis (Fig. 4) show that *Loxoscaphe thecifera* is robustly nested within Aspleniaceae, providing molecular confirmation of the usual contemporary familial placement of this species group. This permits us to take advantage of the recent *rbcL*-based study of the phylogeny of Aspleniaceae by Murakami et al. (1999b) to provide the broadest available molecular framework within which to assess the relationship of *Loxoscaphe* to species of *Asplenium* sensu lato. The results, presented in Figs. 5 and 6, provide insights useful in resolving the conflicting views of contemporary authors regarding generic segregation of *Loxoscaphe* from *Asplenium* summarized above.

The unresolved clade at the top of Fig. 5 is topologically identical to the unresolved uppermost clade in Fig. 1 of Murakami et al. (1999b) where *Asplenium normale* was represented by three varieties. This lack of resolution may be partly attributable to the greatly truncated sequences available for these taxa in GenBank. PCR products generated for these species by Murakami et al. (1999b) contain bases corresponding only to positions 307–1016 of tobacco *rbcL*, as opposed to PCR products corresponding to positions 1–1381 for *Loxoscaphe thecifera* and other taxa when generated with our primers. *Camptosorus sibiricus* (also known as *Asplenium ruprechtii*) is a member of this unresolved clade, providing no information relevant to its recognition as an independent genus. Whether its morphological similarity to American *Camptosorus rhizophyllus* (*Asplenium rhizophyllum*) indicates true phylogeographic vicariance or is homoplastic remains to be tested by molecular data.

The two most basal ingroups in Figs. 5 and 6 correspond to the segregate genera *Phyllitis* and *Hymenasplenium*, as they did in Fig. 1 of Murakami et al. (1999b). *Hymenasplenium* (= *Asplenium* sect. *Hymenasplenium*) has been intensively studied by Murakami and his colleagues (Mitui et al., 1989; Mu-

rakami, 1992, 1995; Murakami and Moran, 1993; Murakami and Schaal, 1994; Cheng and Murakami, 1998; Murakami et al., 1998a, 1998b, 1999a, 1999b). Because it is distinguishable from *Asplenium* morphologically (Murakami and Moran, 1993) and cytologically (Mitui et al., 1989; but note that *H. costarisorum* shares $x = 36$ with *Asplenium* according to Cheng and Murakami, 1998), the deep basal separation of this clade from the rest of *Asplenium* has been accepted by Murakami et al. (1999b) as warranting recognition of *Hymenasplenium* as an independent genus. Similarly, the deep separation of *Phyllitis scolopendrium*, characterized by paired sori whose indusia open toward each other, led Murakami et al. (1999b) to recommend its recognition as an independent genus needing careful morphological redefinition. They did not provide that morphological redefinition, which would seemingly have to overcome Copeland's (1947, pp. 164–165) argument that the soral generic character of *Phyllitis* would be the same as that of *Diplora*, whose position in the *rbcL* phylogeny of Aspleniaceae is unknown.

The large central clade from *Asplenium wrightii* through *Neottopteris nidus* in Figs. 5 and 6 is only weakly supported by four synapomorphies and a bootstrap value of 49%. The clade from *Neottopteris australasica* through *A. prolongatum*, in which *Loxoscaphe* is embedded, is even more weakly supported (four synapomorphies and 37% bootstrap value), but *Loxoscaphe* is confidently placed within the larger clade from *N. australasica* through *N. nidus* with 14 synapomorphies and a 100% bootstrap value. Of the species in this clade, *N. australasica, N. antiqua,* and *N. nidus* were cited by Holttum (1974) as species of *Asplenium* section *Thamnopteris* C. Presl, which is known as *Neottopteris* J. Sm. if given generic rank. Murakami et al. (1999a) differentiated the Japanese specimens of *A. australasicum* (≡ *N. australasica*) sensu Holttum as *A. setoi*; thus the clade from *N. australasica* through *N. nidus* contains four species accepted as members of *Neottopteris* or *Asplenium* section *Thamnopteris*. Murakami et al. (1999b) were surprised by the unexpected sister relationship of *Neottopteris antiqua+nidus* and *Asplenium griffithianum+prolongatum* in their Fig. 1 because *Neottopteris* is defined as having simple leaves in which veins from the midrib run parallel toward the margin, often forking once or less often twice, but with the vein tips always united in a series of short arcs just within the cartilaginous margin of the lamina, the sori being elongate with narrow indusia (Holttum, 1974). *Asplenium prolongatum*, however, has pinnate-pinnatifid leaves that are unique among these four species in having rooting buds at their tips, and *A. griffithianum* has simple leaves whose venation is completely free. Thus *Neottopteris* (*Asplenium* section *Thamnopteris*) as presently defined was paraphyletic in the analysis of Murakami et al. (1999b) and is even more so with *Loxoscaphe* an unexpected and robustly supported member of this clade from *Neottopteris australasica* through *Neottopteris nidus* (Figs. 5, 6).

Although a more comprehensive molecular analysis of the diversity encompassed by *Asplenium* sensu lato is still required, the present study of available *rbcL* data does position *Loxoscaphe* firmly, if enigmatically, within the clade from *Neottopteris australasica* through *N. nidus*. Thus even though eventual

incorporation of *rbcL* data from additional taxa will define clades of other species groups and may modify the relationships of *Loxoscaphe* seen in Figs. 5 and 6, the large number of synapomorphies and 100% bootstrap confidence value with which *Loxoscaphe* is placed within this clade suggest that it will retain its integrity. Moreover, the direct sister relationship of *Loxoscaphe* to *A. griffithianum*+*prolongatum* based on *rbcL* appears to be supported by morphological characters. Robbin C. Moran (pers. comm.) points out that, based on characters of the lamina, *Loxoscaphe thecifera* belongs to a group of primarily African and Madagascan species informally called the "*Darea* group." Among the other species of this group, he lists *A. griffithianum* and *A. prolongatum*. The monophyletic grouping of these three species by *rbcL* (Figs. 5, 6) supports Moran's view that "*Darea*" is a natural group and suggests that Kramer and Viane (1990, p. 56) may have been misled in regarding "*Darea*" as an artificial group based on superficial resemblance. Finally, based on the evidence in Figs. 5 and 6, *Asplenium* sensu lato would have to be broken into many small and probably ill-defined genera before they and some as yet undefined genus *Asplenium* sensu stricto would not be made paraphyletic by the recognition of *Loxoscaphe* as an independent genus. On the basis of the present study, we therefore conclude that although *Loxoscaphe* is placed with certainty in Aspleniaceaeae (Figs. 4–6), there is presently no phylogenetic justification for its acceptance as an independent genus.

ACTINIOPTERIS.—*Actiniopteris* is an Old World genus of five species (Tryon et al., 1990). It is placed in its own family by Pichi Sermolli (1962, 1963) but is more generally treated within Adiantaceae (Crabbe et al., 1975; Kornaś et al., 1982; Jacobsen, 1983) or Pteridaceae (Tryon and Tryon, 1982; Tryon et al., 1990). Tryon and Tryon (1982) divided Pteridaceae directly into tribes and listed *Actiniopteris* and *Onychium* as Old World elements of tribe Cheilantheae (cheilanthoid ferns), not tribe Taenitideae (taenitidoid ferns). The rationale for placing *Actiniopteris* and *Onychium* in the cheilanthoids was not discussed in this book focusing on tropical American ferns, but the authors did note that "The cheilanthoid ferns have been the most contentious group with regard to a practical and natural generic classification" (Tryon and Tryon, 1982, p. 248). In their subsequent taxonomic treatment of Pteridaceae (Tryon et al., 1990), these authors placed *Actiniopteris* and *Onychium* into subfamily Taenitidoideae without explicitly stating their reason for this transfer from the cheilanthoids, but implicating spore morphology in the decision. Their key to subfamilies (1990, pages 231–232) first separates taenitidoids from cheilanthoids on the basis of scales present on the stems in cheilanthoids versus absent in many taenitidoids and secondly on the basis of an equatorial flange present on the spores of taenitidoids that have scales on the stem versus spores without an equatorial flange in cheilanthoids. In this regard these authors were influenced (A. F. Tryon and R. M. Tyryon, pers. comm.) by work already completed for the book on spores of pteridophytes (Tryon and Lugardon, 1991). On page 142 of that work the authors noted that "The arrangement of the flange and ridges in *Onychium* spores is characteristic of the Taenitioid [sic] genera." Tryon and Lugardon (1991) also likened the equa-

torial flange on the spores of *Actiniopteris* to the situation in the taenitidoid genus *Anogramma* and in *Pteris*.

In the parsimony analyses of Hasebe et al. (1995, Fig. 3) the relationships among *Pteris, Onychium,* and *Platyzoma + Taenitis* were unresolved as a polytomy. Our analysis used these same *rbcL* sequences plus those of taenitidoids *Anogramma lorentzii, Pityrogramma calomelanos,* and *P. trifoliata,* an additional species of *Onychium (O. lucidum),* and an additional species of *Pteris* (*P. cretica*). In our analysis (Fig. 7), *Actiniopteris* is robustly grouped with *Onychium* (17 synapomorphies, 99% bootstrap), but the relationships between *Actiniopteris + Onychium* and the other taenitidoid genera and *Platyzoma* are only weakly resolved, with some bootstrap values as low as 22% to 53%. The eventual inclusion of more taenitidoid taxa will probably refine and add confidence to inferred relationships among taenitidoids and between them and *Platyzoma*. At deeper levels in this upper part of Fig. 7, however, the clades from *Platyzoma* through *Pteris* and from *Platyzoma* through *Acrostichum,* within both of which the clade of *Actiniopteris + Onychium* is deeply embedded, are very strongly supported and deeply separated from the well defined clade of cheilanthoids (*Argyrochosma* through *Bommeria*). The present study therefore strongly supports evidence from spore morphology (Tryon and Lugardon, 1991) that *Actiniopteris* and *Onychium* are not cheilanthoids and that the strongest phylogenetic relationships of *Actiniopteris* and *Onychium* are probably with Pteridaceae subfamily Taenitidoideae as hypothesized by Tryon et al. (1990).

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